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A Novel Anisotropic Supramolecular Hydrogel with High Stability over a Wide pH Range

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Abstract

The hydrolysis of carboxylic ester bond, by a base or catalyzed by an enzyme at weak basic condition, serves as the only path to obtain a novel anisotropic supramolecular hydrogel that is stable over a wide pH range. This result not only expands the molecular scope of supramolecular hydrogelators, but also illustrates the design principles for creating pH stable supramolecular soft materials.

Keywords

Supramolecular hydrogel; self-assembly; nanofibers; enzymatic hydrogelation; anisotropic

Supramolecular hydrogels, resulted from the self-assembly of certain small organic molecules driven by non-covalent interactions, have emerged as a type of versatile soft materials and found applications in many areas.¹ Due to their inherent and excellent biocompatibility and biodegradability, supramolecular hydrogels are showing promises to become a useful alternative to polymeric hydrogels.² For example, supramolecular hydrogels are being explored to serve as scaffolds for regenerative medicine,³ wound healing⁴ and biomineralization,⁵ vehicles for controlled drug release,⁶ matrices for protein microarray,⁷ a low cost platform for screening enzyme inhibitors or enzymes detection,⁸ and components for enzyme mimetics.⁹

Forming a hydrogel is the first step for develop supramolecular hydrogels as useful soft materials. There are several different ways to generate supramolecular hydrogels. The commonly used method involves dissolving the hydrogelators into an aqueous solution and changing temperature, pH, or ionic strength to initiate molecular self-assembly in water and result in hydrogelation.¹ This kind of approach, though working well for most of hydrogelators, has some inherent disadvantages for certain hydrogelators, for example, the hydrogelator having exceedingly low solubility (or the hydrophobicity of the hydrogelator is unusually high) or having potential to form precipitates instead of a hydrogel due to the change of temperature, ionic strength, or pH. These molecules have the potential to self-assemble in water despite their poor aqueous solubility. One approach is to dissolve them in a polar organic solvent and then mix with water to form nanostructures or hydrogels. For example, in the works on the self-assembly or hydrogelation of NH₂-Phe-Phe-COOH,¹⁰ Fmoc-Phe-Phe,¹¹ Fmoc-Phe(F5),¹² GSH-pyrene,¹³ and NH⁺₃-Phe-Phe-CO-NH₂,¹⁴ an

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Supporting Information Available: The experiment details, NMR, hydrolysis product analysis, hydrolysis rate, CGC test, dynamic time sweep, video of fast gelation, swelling test, and the normalized intensities of CD. This material is available free of charge via the Internet at <http://pubs.acs.org>.

organic solvent (usually 1,1,1,3,3,3-hexafluoro-2-propanol or DMSO) is always necessary to assist the dissolution of these compounds. Despite its effectiveness, that approach inevitably brings small amount of organic solvent into the hydrogels, which changes, if not completely disturbs, the biocompatibility or rheological behavior of the resulted hydrogels.

To explore new methods for making hydrogels, we and the others have been developing new ways to induce hydrogelation via chemical or enzymatic conversions. For example, the phosphorylation of tyrosine residues on small molecules offers precursors that are soluble at physiological pH. Then a phosphatase can hydrolyze the phosphoric monoester and convert the precursors to less soluble but amphiphilic hydrogelators, which self-assemble in water to form supramolecular nanofibers and result in hydrogels.¹⁵ Besides the route of dephosphorylation, other chemical or enzymatic paths should also be suitable for the generation of supramolecular hydrogels, which are less explored.¹⁶

In this work, we explored the paths for generating the hydrogel of a small molecule (**2**). Due to its high hydrophobicity, neither the change of pH nor the change of temperature creates the hydrogel of **2**. However, a simple chemical modification of **2** offered the molecule **3** (i.e., the precursor of **2**) with excellent solubility at physiological pH. And a chemical (i.e., a strong base) or a biological catalyst (i.e., an esterase) triggered the hydrolysis of the carboxylic ester bond of **3** to produce **2**, which self-assembled into supramolecular hydrogel. Because **2** lacks a carboxylic acid group or an amine group, the hydrogel of **2** exhibits extraordinary stability over a wide pH range, which is crucial for some applications of biomaterials.¹⁷ In addition, unlike most of the supramolecular hydrogels, the hydrogel of **2** is anisotropic (i.e., exhibiting birefringence), which associates with the order of the nanofibers. Besides the identification of a novel hydrogelator, this work illustrates a powerful strategy for evaluating the ability of hydrophobic molecules to form supramolecular hydrogels, which may provide a new direction for designing supramolecular materials based on small molecule therapeutics since a large amount of them are quite hydrophobic.

Figure 1 shows the structures and the synthesis of **2** and **3**. Two cycles of activation of **1** by N-hydroxysuccinimide (NHS) and coupling with the amine group on phenylalanine and ethanolamine, respectively, give **2** in 91% yield (Scheme S1). Finally, the reaction between succinic anhydride and **2** results in **3**, which exhibits drastically increased aqueous solubility (> 20 mg/mL) comparing to that of **2** (< 20 μ g/mL). After obtaining the pure compounds of **2** and **3**, we tested the ability of **2** to form a hydrogel and found that **2** failed to form a hydrogel by changing temperature or adjusting pH. Unlike **2**, compound **3** dissolves easily in a weak basic solution (pH = 7.5) at the concentration of 0.8% (w/v). The addition of small amount of base (NaOH, 1.0 M, 10 μ L) to reach pH = 9 or esterase (1 U/ μ L, 10 μ L) in 400 μ L of the solution of **3** causes the hydrolysis of **3**. The loss of the succinic acid from **3** affords **2** in almost quantitative yield (Figure S1), which self-assembles in water to result a clear hydrogel that shows strong birefringence (Figure 1B), without noticeable pH change. The use of NaOH to hydrolyze **3** helps to determine the hydrolysis rate (Figure S2) and to estimate the critical gelation concentration (CGC) of **2** to be 0.16% (w/v) (Figure S3). We designate the hydrogels formed by the addition of NaOH and esterase as gel **I** and gel **II**, respectively.

We used dynamic time sweep experiment to determine the gelling point upon the addition of the base or the enzyme. The gelling points of gel **I** and gel **II** were about 1 and 38 hours, respectively (Figure S4), which match with the observation in the TEM images of the two hydrogels (Figure 2). According to the TEM results, gel **I**, which formed faster, consists of short fibers (< 500 nm) with more randomly interwoven networks (Figure 2A). Gel **II**,

which formed slower, containing long fibers ($> 2\text{--}3\ \mu\text{m}$) with less dense networks (Figure 2B).

In addition, the increase of the amount of reagents for ester bond hydrolysis can speed up the hydrolysis and thus gelation. Upon the addition of a larger amount of NaOH, gel **I** forms within 3 minutes (see video in the supporting information). Besides controlling the gelation speed, the different routes of hydrolysis may be applied to different applications. For example, the gelation catalyzed by esterase can proceed at physiological condition, which will be useful to encapsulate biomacromolecules. Moreover, we can tailor the properties of the hydrogels with different microstructures for different applications by adding different catalysts at various quantities.

When being immersed in both high acidic and basic solutions, the hydrogels of **2** showed excellent stability (Figure 3A). To further understand the stability of the hydrogel of **2**, we quantified its rheological properties after the gels being treated with HCl (1.0 M) or NaOH (1.0 M). The dynamic strain sweeps and dynamic frequency sweeps reveal that the aged gel **I** preserve its rheological properties after immersed in various pH solutions (20 mL) and a salt solution (20 mL) for half an hour (Figure 3B and 3C). The gel in basic solution (pH = 14) still maintains the G' and G'' that are comparable to the native gel **I**, although the critical strain seemed to decrease from 40% to 4%. The acid-treated gel shows the increase of storage modulus, up to at least 2.5 times of the G' of the native gel **I**. Besides, upon the treatment of 1.0 M HCl solution, the volume of gel **I** changes little (Figure S5). On the other hand, Gel **I** treated by 1M NaCl aqueous solution exhibits a slightly decrease of the viscoelasticity. The above results suggest that the acid may increase the hydrogen bonding among the hydrogelators and water.

Because the formation of gel **II** is slow enough to allow us to monitor the optical and spectroscopic changes over time, we synchronized the time-dependent CD spectra and the time-dependent birefringence of gel **II** to examine the gel formation process (Figure 4). In the first 12 hours, the CD data showed no peaks below 210 nm, neither did we observe birefringence. However, after that, the birefringence, the negative peak at around 209.6 nm and the positive peak at 195.8 nm appeared at the same time. Afterwards, the birefringence increases and those two peaks become bigger and shaper. According to Boden and coworkers' pioneering work,¹⁸ the appearance of these two peaks correspond to the formation of β -sheet. Therefore, these results suggest that the birefringence associate the formation of β -sheet (Figure 4 and Figure S6).

In conclusion, we have demonstrated that the controlled hydrolysis of carboxylic ester bond by a base or an enzyme that can act as a simple trigger to initiate self-assembly and form supramolecular hydrogels, even if the molecule itself is unable to form hydrogels by directly changing pH or temperature. The replacement of carboxylate group with a hydroxyl group on the hydrogelator not only results in a supramolecular hydrogel that is stable over a wide pH range, but also offers a soft supramolecular material that is insensitive to ionic strength. These properties may be particularly useful for design a robust drug release system that can maintain constant release rate against abrupt changes in environment, which is a subject currently under investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) The synthetic route to **2** and **3**. (B) The reactions convert the precursor (**3**) to the hydrogelator (**2**) at basic condition (pH = 9) to afford gel **I** or via hydrolysis catalyzed by esterase (1 U/ μ L, 10 μ L) at pH = 7.5 to give gel **II** (optical images: left, normal; right through a pair of crossed polarizers).

Figure 2.
TEM images of the matrices in (A) gel **I** and (B) gel **II** (aged for seven days).

Figure 3.

(A) Optical images of the gels of **2** immersed in solution with different pHs. (B) Dynamic strain sweeps (at 6.28 rad/s) of aged gels **I** (400 μ L, 0.8 % (w/v)), without treatment and immersed in 1.0 M HCl (20 mL), 1.0 M NaOH (20 mL) and 1.0 M NaCl (20mL) for half an hour. (C) Dynamic frequency sweeps of the corresponding gels **I**.¹⁹

Figure 4.

(A) Optical images of gel **II** (formed by the catalysis of esterase) under crossed polarized light at different time points and (B) the corresponding CD spectra.